Title
Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition

Journal
Biogeochemistry., 117(1)

ISSN
0168-2563

Authors
Kivlin, Stephanie N
Treseder, Kathleen K

Publication Date
2014

DOI
10.1007/s10533-013-9852-2

Biogeochemistry (2014) 117:23–37
DOI 10.1007/s10533-013-9852-2

Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition

Stephanie N. Kivlin · Kathleen K. Treseder

Received: 1 November 2012 / Accepted: 13 April 2013 / Published online: 20 April 2013

Abstract Soil extracellular enzymes are the proximal drivers of decomposition. However, the relative influence of climate, soil nutrients and edaphic factors compared to microbial community composition on extracellular enzyme activities (EEA) is poorly resolved. Determining the relative effects of these factors on soil EEA is critical since changes in climate and microbial species composition may have large impacts on decomposition. We measured EEA from five sites during the growing season in March and 17 sites during the dry season in July throughout southern California and simultaneously collected data on climate, soil nutrients, soil edaphic factors and fungal community composition. The concentration of carbon and nitrogen in the soil and soil pH were most related to hydrolytic EEA. Conversely, oxidative EEA was mostly related to mean annual precipitation. Fungal community composition was not correlated with EEA at the species, genus, family or order levels. The hyphal length of fungi was correlated with EEA during the growing season while relative abundance of taxa within fungal phyla, in particular Chytridiomycota, was correlated with the EEA of beta-glucosidase, cellobiohydrolase, acid phosphatase and beta-xylosidase in the dry season. Overall, in the dry season, 35.3 % of the variation in all enzyme activities was accounted for by abiotic variables, while fungal composition accounted for 27.4 %. Because global change is expected to alter precipitation regimes and increase nitrogen deposition in soils, EEA may be affected, with consequences for decomposition.

Keywords 454 pyrosequencing · Climate · Deserts · Mediterranean ecosystems · Soil nutrients · Soil pH

Introduction

Extracellular enzymes are the proximal drivers of decomposition in soils (Sinsabaugh et al. 2008). Traditionally, soil extracellular enzyme activity (EEA) has been explained by correlations with abiotic factors (Sinsabaugh et al. 2008); however, because soil fungi are responsible for a large portion of enzyme production (Schneider et al. 2012), they may also play a role in determining soil EEA. The relative influence of abiotic factors such as climate, soil pH, and soil nutrient concentrations compared to fungal community composition on EEA is unknown. Understanding

Electronic supplementary material The online version of this article (doi:10.1007/s10533-013-9852-2) contains supplementary material, which is available to authorized users.

S. N. Kivlin · K. K. Treseder
Ecology and Evolutionary Biology, University of California Irvine, Irvine, CA 92697, USA

S. N. Kivlin (✉)
Section of Integrative Biology, Patterson Labs, University of Texas at Austin, Austin, TX 78712, USA
e-mail: stephanie.kivlin@utexas.edu

the drivers of EEA is critical, as EEA can impact decomposition in current and future climates and global change conditions.

Correlations between abiotic factors and soil EEA have been examined more thoroughly than the relationships between microbial communities and EEA. For example, in a synthesis of 40 studies, EEA was largely affected by soil organic matter content, soil pH, and climate (Sinsabaugh et al. 2008). Soil nutrient and carbon (C) concentrations may affect soil EEA through a variety of mechanisms. Stoichiometric constraints on soil microorganism biomass (Cleveland and Liptzin 2007) may influence EEA, such that soil microorganisms produce enzymes to target the most limiting nutrient (Allison and Vitousek 2005; Sinsabaugh and Moorhead 1994). Alternatively, soil C and nutrient stoichiometry may predict EEA based on microbial demands (Sinsabaugh et al. 2009), such that EEA of C-acquiring enzymes is positively correlated with nitrogen (N) and phosphorus (P) acquiring enzymes in a 1:1:1 ratio. Soil pH can also have large impacts on EEA. Extracellular enzymes have pH optima where the active site is in the most operative conformation (Leprince and Quiquampoix 1996). For example, glycosidases have an optimal pH of $\sim$5, whereas polyphenol oxidase and peroxidase have their highest activity at a pH of $\sim$8 (Frankenberger and Johanson 1982).

Climate may also influence EEA. Enzyme activities normally increase with higher temperatures up to an optimum >40 °C (Stone et al. 2012). Similarly, EEA can be affected by soil water concentrations via changes in the diffusion rate of substrates and inhibitory compounds (Zak et al. 1999; Toberman et al. 2008). At global scales, temperature may be more influential than moisture in determining EEA (German et al. 2012); at regional scales, however, changes in soil moisture may become more important. For example, in a study across seven forests, Brockett et al. (2012) found that soil moisture was the most consistent predictor of both hydrolytic and oxidative EEA.

Soil EEA may also vary seasonally. In southern California, soil moisture and nutrient concentrations can vary two-fold between the growing and dry season (Parker and Schimel 2011). Plants can also act as priming agents to stimulate EEA production during the growing season (Averill and Finzi 2011). Taken together, this evidence predicts that soil EEA should be highest during the growing season and lowest during the dry season. However, synthesis based on previous studies remains enigmatic. While some evidence supports seasonal variability in EEA that corresponds to C and nutrient availability (Boerner et al. 2005; Wallenstein et al. 2009), other studies have shown the EEA can actually increase in the nutrient-poor dry seasons (Parker and Schimel 2011), which has been suggested as a microbial increase in EEA production in anticipation of rain events. Still other studies observe no seasonality in EEA (Choi et al. 2009).

While the impact of climatic and soil factors on soil EEA have received much attention, the role of microbial community composition on EEA is less well understood. Recent meta-genomic and proteomic studies have shown that fungi produce the majority of extracellular enzymes in litter and soil that degrade labile and recalcitrant C, organic N, and organic P polymers (Schneider et al. 2012, 2010). This supports previous research showing that soil fungi comprise a significant portion of microbial biomass belowground (Joergensen and Wichern 2008; Strickland and Rousk 2010) and regulate C and nutrient cycling in terrestrial ecosystems (Hattenschwiler et al. 2005). Numerous studies have found significant correlations between phospholipid fatty acid (PLFA) profiles and EEA (DeForest et al. 2012; Waldrop et al. 2000; Brockett et al. 2012; Kourtev et al. 2003), but a mechanistic understanding of how EEA may change among fungal groups is still unclear. Soil EEA may also be influenced by microbial groups that are not delineated by their fatty acid profiles. For example, soil decomposer communities may impact EEA if different functional groups produce disparate suites of enzymes, (i.e., opportunists, decomposers, and miners (Moorhead and Sinsabaugh 2006). Moreover, oxidative and hydrolytic enzymes may be produced by different fungal phyla, as the abundance of Basidiomycota is often positively correlated with the activity of oxidative enzymes (Frey et al. 2004). While correlations between fungal PLFA profiles and EEA are well documented, the relationships between fine-scale fungal community composition and EEA have not been directly examined in natural systems. Disen-

tangling the relative influence of abiotic factors and fungal composition on EEA remains difficult as fungal composition varies over geographic space and between habitats (Lauber et al. 2008). Therefore the environment may indirectly influence EEA through shifts in fungal community composition. Indeed, in recent global syntheses, fungal community composition in soils was largely affected by climate and soil edaphic factors (Tedersoo et al. 2012; Kivlin et al. 2011).

Here, we specifically examined the correlations between abiotic factors, fungal community composition and soil EEA by analyzing EEA in response to both factors (abiotic v. fungal composition) separately and concurrently across a variety of ecosystems. A subset of enzymes and sites were measured at two time points, March and July, to understand how seasonality may affect EEA. We predicted that all EEA would be positively correlated to soil nutrient concentrations, if nutrient availability controlled enzyme activities (sensu Sinsabaugh et al. 2009, but see Allison and Vitousek 2005). Based on prior analyses (Sinsabaugh et al. 2008), we also expected that all EEA would to be positively correlated with mean annual precipitation (MAP) and soil moisture, as moisture is known to affect all EEA (German et al. 2012). We also hypothesized hydrolytic EEA would be negatively correlated with soil pH if it largely influenced enzyme active site conformation. Most hydrolytic active sites are optimal around pH of 5 (Frankenberger and Johanson 1982). Conversely, we expected oxidative EEA to be positively correlated with soil pH, since these enzymes have active sites with optimal configurations at pH $\sim$8 (Frankenberger and Johanson 1982). We further hypothesized that enzyme activities would vary with fungal community composition if different fungal groups produced different concentrations and/or classes of enzymes (C-degrading v. P-degrading). In particular, we predicted that the relative abundance of Glomeromycota fungi would be positively correlated with P-degrading EEA, while the relative abundance of Ascomycota and Basidiomycota was expected to be positively correlated with all EEA, as this trend is often observed in other systems (Frey et al. 2004). Finally, we anticipated abiotic factors to be more related to EEA than fungal composition since we expected fungal community composition itself to be affected by abiotic factors.

Materials and methods

We collected five 2.5 $\times$ 10 cm soil cores in March 2010 from five sites and in July 2010 from 17 sites throughout southern California, between 34.61°N, 120.23°W; 34.15°N, 116.46°W; and 33.46°N, 117.04°W (Table 1). Our sites included a variety of ecosystems (grasslands, scrublands, deserts and forests) over a spatial scale large enough to vary in climate and soil parameters, but small enough to potentially observe fungal dispersal between sites (Brown and Hovmoeller 2002; Peay et al. 2012). At each site, we collected the following abiotic variables: soil pH, soil C and N, soil NH_4^+ and NO_3^-, soil PO_4^{3-}, soil moisture, MAP, latitude, longitude, and elevation. We also assayed fungal community composition at the species, genus, family and order level of resolution, and as differences between the relative abundance of each phylum. A subsample of soil was processed to determine soil pH with a 1:1 ratio (w/v) of soil to diH_2O. Another subsample was acidified and then combusted to determine total soil C, N and the C:N ratio on a Thermo Finnigan IRMS at the UCI IRMS facility (Robertson et al. 1999). We determined soil moisture gravimetrically and soil ammonium, nitrate and phosphate concentrations via resin extraction and colorimetric assays (Robertson et al. 1999). Resin extractions were performed on field soils in the laboratory, by shaking 10 g of soil in 50 ml diH_2O with a mixed resin bag for 18 h. We also measured total fungal abundance by extracting fungal hyphae from 5 g of soil and microscopically counting hyphal abundance via the grid-line intersect method (Brundett et al. 1984). All measurements were conducted in triplicate and pooled for each site at each time point (Table 1).

We measured a broad range of EEA to incorporate enzymes relevant for C, N, and P cycling including hydrolytic and oxidative enzymes. We measured hydrolytic soil EEA for multiple C-degrading enzymes: alpha-glucosidase (AG), which targets byproducts of starch; beta-glucosidase (BG), byproducts of cellulose and glucose; beta-xylosidase (BX), xylose; cellobiohydrolase (CBH), cellulose. We also measured enzymes relevant for N cycling, (*N*-acetyl-glucosaminidase, NAG) and P cycling (acid phosphatase AP). Hydrolytic enzyme activities were determined from approximately 1 g of fresh soil samples using fluorescently-labeled substrates (Allison et al.

Table 1 Site information for all abiotic variables and the abundance of each fungal phylum at each time point. Means of three replicates are presented with standard errors in parentheses

	Latitude	Longitude	Elevation (m)	Vegetation	MAP (cm)	Soil NH_4^+ (ppm)	Soil NO_3^-(ppm)	Soil pH	Soil Moisture
Box (03/10)	33.97	−117.29	527.85	Grass	33.3	30 (0.1)	3 (0.1)	6.4 (0.1)	11.45 (N/A)
Irvine Ranch (3/10)	33.70	−117.58	462.08	Grass	33.0	20 (0.1)	17 (0.5)	6.2 (0.1)	16.44 (N/A)
Motte Reserve (03/10)	33.81	−117.26	543.89	Grass	30.4	34 (0.1)	2 (0.1)	6.3 (0.1)	10.61 (N/A)
Santa Cruz Reserve (03/10)	34.01	−119.58	69.68	Grass	55.6	25 (0.1)	7 (1.0)	6.5 (0.1)	19.26 (N/A)
Sedgwick Reserve (03/10)	34.68	−120.09	384.78	Grass	54.5	74 (0.3)	7 (0.5)	6.1 (0.1)	24.72 (8.74)
Backbay (07/10)	33.65	−117.87	10.42	Scrub	31.3	44 (16)	1 (0.1)	6.7 (0.2)	0.84 (0.08)
Box Reserve (07/10)	33.97	−117.29	527.85	Grass	33.3	17 (5)	5 (0.3)	7.7 (0.1)	0.44 (0.02)
Boyd Chaparral (07/10)	33.61	−116.46	1,312.59	Desert	35.8	14 (6)	1 (0.1)	7.9 (0.1)	0.10 (0.03)
Boyd Pinyon (07/10)	33.61	−116.46	1,270.47	Forest	35.8	33 (10)	1 (0.1)	7.8 (0.1)	0.29 (0.03)
Burns Reserve (07/10)	34.14	−116.44	1,256.81	Desert	25.5	16 (7)	2 (0.1)	7.6 (0.1)	0.21 (0.01)
Carpenteria Reserve (07/10)	34.40	−119.54	1.00	Scrub	41.1	53 (4)	3 (0.4)	7.8 (0.1)	0.90 (0.04)
Crystal Cove SP (07/10)	33.58	−117.85	26.00	Scrub	31.5	78 (5)	5 (0.7)	7.7 (0.1)	3.01 (0.05)
Gaviota SP (07/10)	34.49	−120.21	1.00	Scrub	58.9	8 (6)	4 (0.3)	7.7 (0.1)	0.84 (0.21)
James Reserve (07/10)	33.79	−116.80	1,325.00	Forest	59.6	3 (2)	2 (0.2)	7.2 (0.1)	1.97 (0.73)
Motte Reserve (07/10)	33.81	−117.26	543.89	Grass	30.4	23 (6)	4 (0.1)	7.3 (0.1)	1.29 (0.76)
Pt.Mugu SP (07/10)	34.01	−118.82	7.00	Scrub	36.8	37 (27)	2 (0.5)	7.7 (0.1)	6.70 (3.63)
San Joaquin Reserve (07/10)	33.66	−117.85	3.54	Scrub	32.0	61 (18)	4 (0.3)	7.4 (0.2)	1.36 (0.06)
San Onofre SP (07/10)	33.39	−117.59	35.00	Scrub	29.5	41 (13)	6 (0.5)	6.9 (0.2)	1.94 (0.28)
Santa Cruz Reserve (07/10)	34.01	−119.58	69.68	Grass	55.6	2 (1)	3 (0.1)	7.2 (0.1)	3.05 (0.33)
Sedgwick Reserve (07/10)	34.68	−120.09	384.78	Grass	54.5	32 (9)	4 (0.3)	7.1 (0.1)	2.66 (0.08)
Stunt Reserve (07/10)	34.10	−118.64	448.06	Forest	65.8	44 (1)	3 (0.3)	7.4 (0.1)	4.25 (0.10)
Topanga Canyon SP (07/10)	34.10	−118.60	279.00	Scrub	59.0	14 (5)	7 (0.3)	7.1 (0.1)	2.75 (0.23)

	Available PO_4^{3-}(ppm)	Nitrogen (%)	Carbon (%)	C:N	Hyphae (mm/g dry soil)	Ascomycota	Basidiomycota	Chytridiomycota	Glomeromycota
Box (03/10)	103 (10)	0.09 (0.01)	1.00 (0.02)	11.64 (0.17)	1,741 (5)	799	65	10	4
Irvine Ranch (3/10)	121 (1)	0.12 (0.01)	1.28 (0.07)	10.58 (0.06)	1,483 (1)	584	390	2	1
Motte Reserve (03/10)	160 (5)	0.08 (0.01)	1.02 (0.02)	12.31 (0.18)	1,481 (55)	512	397	1	0
Santa Cruz Reserve (03/10)	181 (2)	0.19 (0.01)	2.13 (0.10)	11.07 (0.11)	2,005 (306)	854	104	0	0
Sedgwick Reserve (03/10)	225 (5)	0.47 (0.06)	7.54 (0.81)	16.21 (0.20)	2,696 (196)	630	240	3	11
Backbay (07/10)	32 (10)	0.16 (0.01)	1.75 (0.11)	11.07 (0.07)	882 (75)	676	150	4	7
Box Reserve (07/10)	66 (16)	0.07 (0.01)	0.87 (0.04)	12.54 (0.93)	1,001 (123)	637	262	4	11
Boyd Chaparral (07/10)	113 (19)	0.05 (0.01)	0.77 (0.04)	17.05 (1.45)	765 (72)	784	157	2	9
Boyd Pinyon (07/10)	107 (34)	0.07 (0.01)	0.58 (0.04)	8.81 (0.77)	1,028 (224)	741	119	7	3
Burns Reserve (07/10)	50 (4)	0.09 (0.07)	0.88 (0.30)	21.82 (8.0)	451 (6)	550	271	22	7
Carpenteria Reserve (07/10)	110 (54)	0.07 (0.01)	0.58 (0.02)	8.58 (0.43)	426 (23)	806	74	10	7

Table 1 continued

	Available PO_4^{3-} (ppm)	Nitrogen (%)	Carbon (%)	C:N	Hyphae (mm/g dry soil)	Ascomycota	Basidiomycota	Chytridiomycota	Glomeromycota
Crystal Cove SP (07/10)	83 (4)	0.17 (0.01)	1.90 (0.05)	11.23 (0.08)	1,109 (185)	408	317	10	5
Gaviota SP (07/10)	213 (24)	0.10 (0.01)	1.28 (0.03)	12.41 (0.17)	1,561 (99)	765	64	16	17
James Reserve (07/10)	30 (7)	0.16 (0.02)	4.37 (0.36)	27.54 (0.63)	4,973 (156)	739	82	7	10
Motte Reserve (07/10)	70 (18)	0.10 (0.01)	1.20 (0.03)	12.54 (0.90)	823 (139)	638	241	10	6
Pt.Mugu SP (07/10)	73 (16)	0.19 (0.01)	2.50 (0.23)	13.29 (0.38)	1,201 (182)	646	226	6	20
San Joaquin Reserve (07/10)	90 (7)	0.22 (0.01)	2.81 (0.20)	12.83 (0.13)	1,089 (153)	442	322	0	2
San Onofre SP (07/10)	70 (20)	0.10 (0.01)	1.07 (0.04)	10.49 (0.64)	1,139 (242)	837	45	11	0
Santa Cruz Reserve (07/10)	34 (4)	0.37 (0.01)	5.31 (0.02)	14.26 (0.19)	1,303 (109)	825	89	5	0
Sedgwick Reserve (07/10)	115 (34)	0.47 (0.01)	5.17 (0.19)	11.06 (0.19)	1,262 (104)	941	33	2	2
Stunt Reserve (07/10)	72 (2)	0.17 (0.01)	0.91 (0.04)	5.52 (0.07)	1,724 (178)	665	218	10	4
Topanga Canyon SP (07/10)	39 (3)	0.26 (0.01)	4.36 (0.12)	16.96 (0.21)	910 (63)	597	207	5	4

2009). We also measured the oxidative enzyme polyphenoloxidase (PPO), which degrades lignin. PPO activities were characterized from ~1 g of fresh soil using the L-3,4-dihydroxyphenylalanine substrate following Allison et al. (2009). All enzymes were measured at the July sampling point, while only CBH, BG, AG and NAG were measured at the March sampling point. All soils were mixed in a sodium acetate buffer (pH 5.5), and incubated for 1 h at 23 °C. Hydrolytic enzymes were measured fluorometrically at an excitation wavelength of 365 nm and emission wavelength of 450 nm, and oxidative enzymes were determined via absorbance at 460 nm.

DNA was extracted in duplicate from ~0.25 g of soil from each site at each time point using the MoBio Power Soil extraction kit and pooled per site. DNA was amplified with conserved fungal primers in the 18S region as detailed in Rousk et al. (2010). Samples were sequenced at the Environmental Genomics Core Facility at the University of South Carolina via a 454 Life Science Genome Sequencer FLX Roche machine. Sequences were quality checked, trimmed to 200 bases and denoised using the default settings in Denoiser (Reeder and Knight 2010). Sequences were clustered into operational taxonomic units (OTUs) at the 92, 95, 97, 98, 98.5, 99, and 100 % similarity cutoffs based on PyNAST aligned and filtered sequences (Caporaso et al. 2010a) using the UCLUST algorithm (Edgar 2010). These cutoff assignments ranged between defining different fungal orders, families, genera, and species. For each sampling site, we rarefied our OTUs to 1,000 sequences to ensure equal sampling effort between sites. Sequences were assigned a taxonomic identity at the phylum level by using the BLASTn algorithm on one representative sequence per OTU against the NCBI database with an expect value of $1e^{-6}$ and deposited in the GenBank sequence read archive (SRA046762.1). The relative abundance of each phylum at each site was calculated as the number of sequences out of 1,000 that returned a positive BLAST hit for each focal phylum. All analyses were implemented using the QIIME pipeline (Caporaso et al. 2010b).

Statistics

The four EEA measured during both seasons (CBH, BG, AG, and NAG) were tested for differences between sampling times with a univariate ANOVA.

Although EEA did not differ between time points for most enzymes (CBH: $F = 0.36$, $P = 0.56$; BG: $F = 12.45$, $P = 0.002$; AG: $F = 3.22$, $P = 0.09$; NAG: $F = 1.399$, $P = 0.25$), we analyzed each time point separately because EEA could potentially be correlated with different abiotic and fungal factors in different seasons.

To analyze if EEA correlated with fungal composition based on taxonomic resolution, we independently regressed EEA for each enzyme at each OTU similarity cutoff for each time point using the Adonis function in the Vegan package in R (R Development Core Team 2009). This approach is similar to distance-based RDA models (Legendre and Anderson 1999). To determine the environmental variables that best explained EEA for each enzyme, we separately regressed each abiotic factor (total soil C, total soil N, soil C:N, soil NO_3^-, soil NH_4^+, soil PO_4^{3-}, soil pH, soil moisture, MAP, latitude and longitude, and elevation) with each EEA at each time point. We also regressed the relative abundance of each fungal phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota), the ratio between all phyla and total fungal biomass against each EEA, in separate single regression models, to determine if fungal composition affected EEA at each time point. We then attempted to improve the model fit created by regressing single variables by creating two stepwise multiple regression models for each sampling time. First we created a model with abiotic variables alone, and then one with fungal composition alone. We used Akaike's information criterion (AIC) (Akaike 1974) to understand how including multiple explanatory variables affected model fit compared to single variable models. Multiple variable models were only reported as significant if they increased AIC model fit compared to single variable models. All variables met the assumptions of normality, except soil moisture, soil PO_4^{3-}, total soil C, total soil N, soil C:N and hyphal lengths, which were all natural-log transformed to improve normality.

Differences in EEA correlated with abiotic variables could be driven directly by abiotic variables or indirectly by shifts in fungal communities mediated by abiotic variables. Additionally, abiotic variables and fungal composition may be spatially structured. We partitioned the relative contribution of abiotic variables, fungal composition and spatial separation to each EEA using dbRDA with the capscale function in the Vegan package in R (R Development Core Team 2009). We only present data for the dbRDA from the July sampling point as the March time point did not contain enough sites to account for the appropriate number of degrees of freedom to run dbRDA. Because abiotic variables and fungal composition are often correlated, we also performed Pearson correlations for all pairwise comparisons for all variables. All P values were adjusted using the Bonferroni correction (Dunn 1961) to account for multiple comparisons. All regression and correlation statistics were calculated in SPSS v. 17.

Results

MAP varied between 25 to 66 cm throughout our sites. Total soil N ranged between 0.05 to 0.47 %; total soil C between 0.58 and 7.54 %; and soil pH 6.1–7.9 (Table 1). Mean annual temperature did not differ between our sites (data not shown) as most sites occurred at similar latitudes. Hydrolytic EEA varied by at least an order of magnitude among our sites, while oxidative EEA varied 6 fold between sampling locations (Table 2).

Abiotic variables and EEA

Soil EEA of all enzymes was positively correlated with the total amount of soil C and soil N at both time points ($P < 0.05$) (Table 3), supporting our hypothesis that soil resources would be correlated with EEA. MAP was positively correlated with the activity of PPO during the dry season. Soil moisture was positively correlated with BG and CBH during the growing season and NAG during the dry season ($P < 0.05$) (Table 3), which partially supported our hypothesis that soil moisture should affect EEA. Soil pH was also negatively correlated with the activity of BG, NAG and AP during the dry season sampling point ($P < 0.05$) (Table 3), partially supporting our hypothesis that hydrolytic enzyme activities would be negatively correlated with soil pH. Multiple regression models using only abiotic variables only improved model fit for AP activity during the dry season, which was explained by the concentration of soil N and soil pH (Tables 3, S1). Inorganic soil nutrients were rarely related to enzyme activities. Only soil NH_4^+ and PO_4^{-3} were related to EEA during the growing season, while

Table 2 Enzyme activities of each enzyme at each site and time point with standard errors in parentheses

Site	BG		CBH		AG		NAG		AP		BX		PPO	
Box Reserve (03/10)	2,996	(163)	446	(38)	67	(2)	365	(34)	N/A	N/A	N/A	N/A	N/A	N/A
Irvine Ranch (03/10)	2,547	(205)	413	(37)	63	(5)	634	(40)	N/A	N/A	N/A	N/A	N/A	N/A
Motte Reserve (03/10)	2,098	(88)	323	(281)	61	(9)	382	(19)	N/A	N/A	N/A	N/A	N/A	N/A
Santa Cruz Reserve (03/10)	7,690	(579)	764	(84)	88	(29)	824	(78)	N/A	N/A	N/A	N/A	N/A	N/A
Sedgwick Reserve (03/10)	9,972	(274)	2,289	(86)	263	(27)	2,475	(60)	N/A	N/A	N/A	N/A	N/A	N/A
Backbay (07/10)	2,138	(127)	249	(13)	184	(10)	960	(61)	3,727	(258)	305	(23)	255	(3)
Box Reserve (07/10)	2,451	(118)	2,264	(196)	265	(4)	732	(107)	1,846	(200)	348	(16)	361	(9)
Boyd Pinyon Reserve (07/10)	970	(58)	115	(11)	138	(4)	221	(14)	1,383	(258)	189	(23)	269	(5)
Boyd Shrub Reserve (07/10)	930	(70)	130	(17)	131	(10)	186	(18)	697	(258)	173	(23)	151	(5)
Burns Reserve (07/10)	301	(15)	30	(2)	38	(2)	54	(6)	358	(13)	52	(2)	107	(1)
Carpenteria Reserve (07/10)	867	(46)	124	(19)	108	(4)	206	(20)	84	(20)	145	(8)	418	(16)
Crystal Cove SP (07/10)	2,145	(37)	421	(14)	201	(7)	702	(19)	1,601	(44)	210	(12)	250	(17)
Gaviota SP (07/10)	2,191	(67)	264	(14)	226	(6)	394	(77)	315	(30)	254	(14)	323	(8)
James Reserve (07/10)	1,341	(55)	182	(24)	114	(12)	1,019	(60)	2,625	(166)	195	(22)	305	(6)
Motte Reserve (07/10)	1,943	(77)	1,814	(45)	223	(8)	652	(23)	1,828	(67)	305	(8)	355	(42)
Pt. Mugo SP (07/10)	1,508	(89)	1,635	(59)	206	(7)	468	(53)	1,812	(172)	241	(6)	203	(7)
San Joaquin Reserve (07/10)	2,391	(109)	2,415	(137)	182	(5)	717	(46)	2,575	(163)	289	(16)	193	(5)
San Onofre SP (07/10)	1,832	(106)	257	(17)	149	(4)	851	(27)	2,966	(81)	245	(13)	217	(3)
Santa Cruz Reserve (07/10)	3,512	(204)	3,344	(92)	360	(13)	1,241	(32)	4,184	(89)	509	(16)	593	(33)
Sedgwick Reserve (07/10)	3,692	(254)	2,928	(203)	395	(20)	1,119	(70)	5,010	(183)	588	(80)	609	(9)
Stunt Reserve (07/10)	1,346	(49)	130	(8)	146	(4)	477	(34)	1,856	(46)	160	(9)	435	(6)
Topanga SP (07/10)	2,215	(120)	344	(23)	151	(7)	767	(66)	1,886	(174)	205	(27)	585	(16)

SP state park

Table 3 Regression models of abiotic factors affecting EEA at each time point

	Soil C	Soil N	Soil NH_4^+	Soil PO_4^{3-}	Soil pH	Soil moisture	MAP	Elevation	Latitude	Longitude	Multiple
BG (03/10)	0.94	0.90	–	–	–	0.89	0.97	–	–	−0.99*	–
CBH (03/10)	0.99*	0.98	–	–	–	0.88	–	–	0.95	−0.91	–
AG (03/10)	0.97*	0.93	0.91	–	–	–	–	–	0.97	–	–
NAG (03/10)	0.99*	0.96	0.91	–	–	–	–	–	0.93	–	–
BG (07/10)	0.68	0.73*	–	–	−0.50	–	–	−0.54	–	−0.58	–
CBH (07/10)	0.56*	0.55	–	–	–	–	–	−0.48	–	–	–
AG (07/10)	0.54	0.60*	–	–	–	–	–	–	–	−0.59	–
NAG (07/10)	0.79*	0.74	–	−0.50	−0.78	0.58	–	–	–	–	–
AP (07/10)	0.70	0.75*	–	–	−0.78*	–	–	–	–	–	0.86
BX (07/10)	0.59	0.63*	–	–	–	–	–	–	–	−0.51	–
PPO (07/10)	0.49	0.59	–	–	–	–	0.70*	–	0.57	−0.66	–

Each individual regression (r) is presented when $P < 0.05$. All variables that are significant at $P < 0.05$ in the multiple regression model are starred with an asterisk

soil PO_4^{-3} was related to EEA during the dry season ($P < 0.05$). Overall, most abiotic variables were not significantly correlated with each other. However, the concentrations of total soil C and total soil N were significantly positively correlated at both time points ($P < 0.001$) (Tables S2, S3).

Fungal composition and EEA

Extracellular enzyme activities were never correlated with fungal composition at any OTU cutoff in our adonis models (Table S4). However, the EEA of BG, CBH, and NAG increased with hyphal length during the growing season. The relative abundance of Glomeromycota was also positively correlated with AG activity during the growing season. Activities of BG, CBH, NAG, AP, and BX decreased significantly as Chytridiomycota became more abundant in single regression models during the dry season ($P < 0.05$) (Table 4). Furthermore, in single regressions, the ratios of Ascomycota:Basidiomycota and Ascomycota:Chytridiomycota were positively correlated with the activity of BX in the dry season ($P < 0.05$) (Table 4). Finally, during the dry season the ratio of Ascomycota:Glomeromycota was significantly positively correlated with the activity of BG, AP, and BX ($P < 0.05$) (Table 4). Multiple regression models improved model fit for AP and BX during the dry season. The model fit of AP activity was predicted best by including the relative abundance of Chytridiomycota and the ratio of Ascomycota:Glomeromycota (Tables 4, S1). The activity of BX was predicted best by including the relative abundance of Chytridiomycota, and the ratio of Ascomycota:Basidiomycota. Contrary to our hypotheses, the relative abundance of Glomeromycota was not related to phosphatase activities and the relative abundance of Ascomycota and Basidiomycota by themselves never explained the activity of any enzyme. The relative abundance of Ascomycota and Basidiomycota were positively correlated (Tables S2, S3).

Combined abiotic variables and fungal composition and EEA

In the dbRDA for the dry season, abiotic factors accounted for 35.3 % of the variation in all enzyme activities (Fig. 1a; Table 5). Fungal composition accounted for 27.4 % of variation in enzyme activities (Fig. 1b; Table 5). Spatial separation accounted for 10.0 % of the variation and 27.3 % of the variation was unaccounted for in our model. The relative contribution of abiotic factors, fungal composition and geographic space varied between enzymes (Table 5).

Discussion

Overall, soil EEA was mostly correlated with abiotic variables. Fungal composition and biomass also correlated with EEA, but to a lesser extent. The

Table 4 Regression models of fungal community factors affecting EEA

	Hyphal length	Chytridiomycota	Glomeromycota	Ascomycota:Basidiomycota	Ascomycota:Chytridiomycota	Ascomycota:Glomeromycota	Multiple
BG (03/10)	0.96*	–	–	–	–	–	–
CBH (03/10)	0.98*	–	–	–	–	–	–
AG (03/10)	0.92	–	0.92*	–	–	–	–
NAG (03/10)	0.90*	–	–	–	–	–	–
BG (07/10)	–	−0.49*	–	–	–	0.49	–
CBH (07/10)	–	−0.59*	–	–	–	–	–
AG (07/10)	–	–	–	–	–	–	–
NAG (07/10)	0.53*	−0.49	–	–	–	–	–
AP (07/10)	–	−0.55*	–	–	–	0.61*	0.77
BX (07/10)	–	−0.53*	–	0.55*	0.52	0.53	0.74
PPO (07/10)	–	–	–	–	–	–	–

Each individual regression (r) is presented when $P < 0.05$. All variables that are significant at $P < 0.05$ in the multiple regression model are starred with an asterisk

relative influence of most abiotic variables and fungal composition differed between seasons while only the abiotic factors of soil C and N consistently correlated with EEA for all enzymes at both sampling points. Sinsabaugh et al. (2008) also found that BG, AP and PPO activities were highly positively correlated to soil organic matter concentrations, suggesting that soil C and nutrient concentrations may be the most universal variable influencing soil EEA at both global and regional scales, and are the most relevant parameters for biogeochemical models.

Soil C and nutrient concentrations can influence EEA by multiple mechanisms. The most likely way that soil C and nutrients impact EEA is by affecting the concentration of available substrate in the soil and the stoichiometry of C, N and P. In a global study, Sinsabaugh et al. (2009) demonstrated that EEA is largely controlled by the stoichiometry of soils and microbial biomass such that C, N and P acquiring enzymes are produced in roughly equal proportions. Soil C and nutrients can also affect enzymes via more indirect mechanisms. For example, increased soil C can often lead to increased water retention (Hudson 1994), which can increase substrate and enzyme diffusion. This mechanism is supported by our data, as MAP was also positively correlated with EEA of PPO. The effects of soil C and nutrient concentrations on EEA may become relevant for biogeochemical models in future global change scenarios. For example, because EEA of all enzymes was correlated with total soil N, and occasionally with inorganic N pools, the predicted increases in N-deposition in the Los Angeles basin (Fenn et al. 2003) may increase the EEA of C, N and P-degrading enzymes. Stimulation of EEA by N-deposition has been observed previously and our findings suggest that this trend may be relevant across the growing and dry seasons in a variety of ecosystems (Saiya-Cork et al. 2002; Henry et al. 2005 but see DeForest et al. 2004).

Soil pH was often correlated with EEA in our sites during the dry season and in multiple sites across North America (Sinsabaugh et al. 2008). Soil pH can affect EEA by changing the conformation of the enzyme active site, so that various enzyme isoforms perform more efficiently at different pHs (Frankenberger and Johanson 1982). This mechanism is particularly relevant for AP, which was strongly correlated with soil pH during the dry season sampling point in our study. Soil pH could also affect EEA

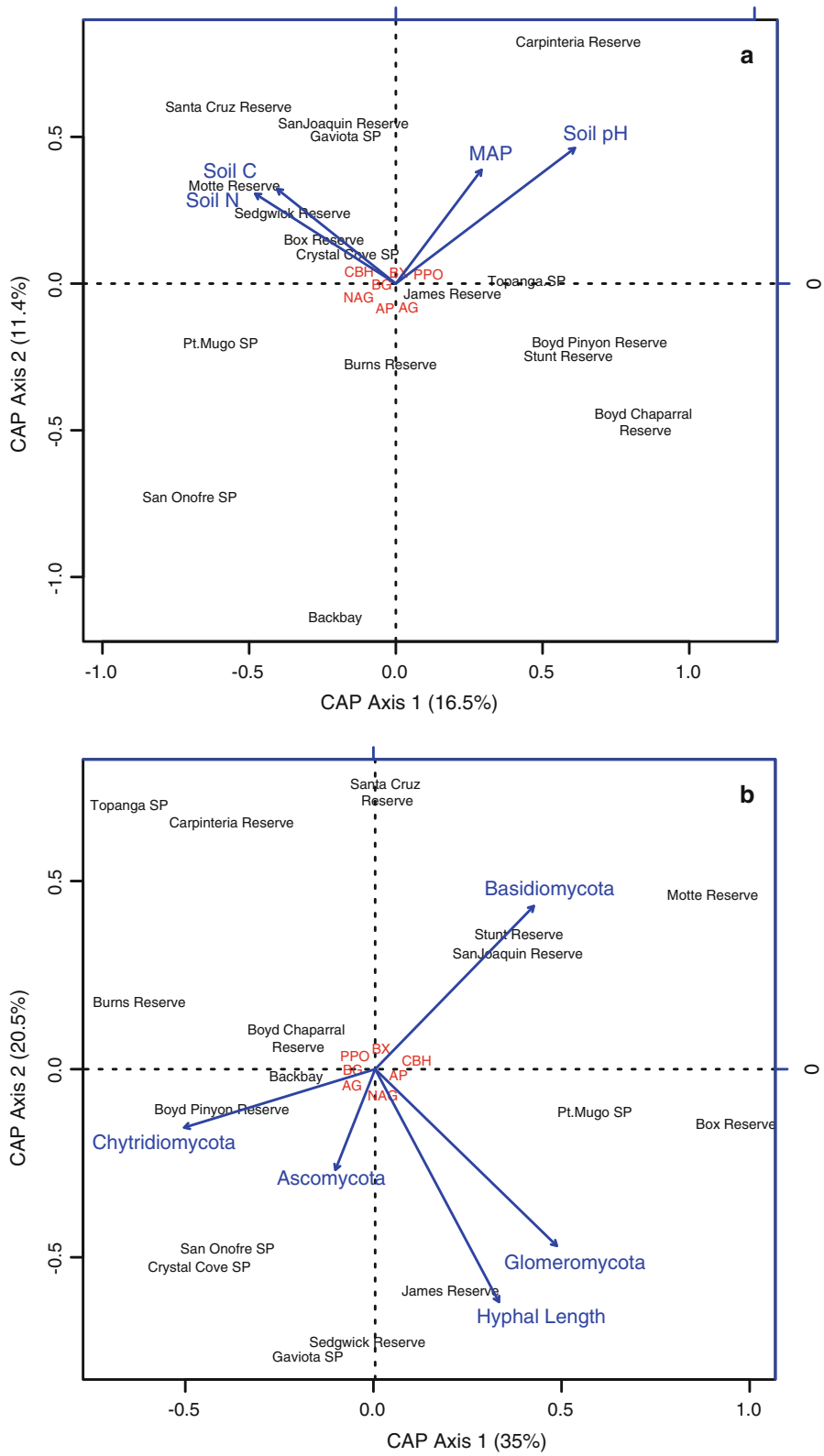

Fig. 1 dbRDA ordination for all abiotic factors without the influence of fungal community composition or spatial separation **a** and fungal composition without the influence of abiotic factors or spatial separation **b** for the dry season sampling date (July). Individual variables in the ordination are with *arrows*

Table 5 Variance from dbRDA explained by abiotic factors, fungal community composition and space and unresolved variance for each enzyme activity and all enzyme activities pooled

Enzyme	Abiotic factors (%)	Fungal composition (%)	Space (%)	Unresolved variance (%)
BG	35.1	28.7	7.8	9.5
CBH	37.4	33.0	10.0	9.8
AG	30.4	26.4	11.5	14.8
NAG	36.3	25.5	4.8	7.3
AP	31.8	24.0	11.9	11.6
BX	39.1	26.9	13.4	11.8
PPO	24.5	19.6	8.1	18.9
All enzymes	35.3	27.4	10.0	10.4

indirectly through changes in soil microbial communities. Soil pH is highly correlated with bacterial (Fierer and Jackson 2006) and fungal community composition (Rousk et al. 2010 but see Lauber et al. 2008), and can be inversely correlated with fungal growth rate (Rousk et al. 2009). Because fungal biomass was positively correlated with EEA in our study, it is unlikely that the negative correlations between soil pH and enzyme activities we observed are explained by this mechanism. However, if bacterial and fungal taxa differ in enzyme production, changes in soil pH may affect soil EEA through modifications in soil microbial communities. Our experimental conditions may have skewed the relationship between soil pH and EEA. Our enzyme assays were conducted in buffer with a pH of 5.5 (the optimal pH for most enzymes we surveyed), while soils were significantly more basic. If EEA in our systems is locally adapted to soil pH (Fernandez-Calvino et al. 2011; Turner 2010), conducting our assays in an acidic environment could have created the negative correlations between EEA and soil pH, which we observed for BG, NAG and AP. Nevertheless, these methodological artifacts may not be the main explanatory driver of our results. If the buffer pH affected EEA, this effect should be consistent between sampling dates and sites; our results do not support this trend. The activities of BG and NAG were not significantly correlated with soil pH during the March sampling date and varied nearly as much (2.5×) at neutral soil pH between sites as they did across all soil pHs during the July sampling date.

None of the enzyme activities was correlated with shifts in fungal community composition at any OTU cutoff, and many were unrelated to the relative abundance of fungal phyla. However, fungal hyphal length was positively correlated with multiple C-degrading enzymes during the growing season. At that time, plants exude more labile C to fungal symbionts (in our study, Glomeromycota) and soil saprotrophs (in our study Ascomycota and Basidiomycota), which may stimulate hyphal growth and enzyme activities via priming (Averill and Finzi 2011). Indeed, we observed a significantly positive correlation between the relative abundance of arbuscular mycorrhizal fungi (AMF) and AG activity, which may be indicative of priming of saprotrophs by AMF (Cheng et al. 2012; Drake et al. 2012). Activities of BG, CBH and NAG were also correlated with fungal biomass more than any other metric of the fungal community during the growing season. This pattern suggests that C priming by plants stimulates growth of saprotrophic fungi and degradation of more recalcitrant C compounds in the soil (i.e. cellulose and chitin) (Talbot and Treseder 2012, but see Weintraub et al. 2007). Plants may also produce more of their own extracellular enzymes during the growing season to directly acquire soil nutrients (Burns 1978). Alternatively, NAG activity may be higher in soils with more fungi, if fungi are selectively competing with each other by degrading one another's hyphal biomass (Baldrian 2008). This mechanism may be prominent throughout the year, as NAG activity also corresponded to hyphal biomass in the dry season. At that time point, fungal composition and biomass explained nearly one-third of the variation in EEA after other variables were partitioned out of the dbRDA model. The relative sequence dominance of the Chytridiomycota was related to EEA the most. However, the relative dominance of Chytridiomycota fungal sequences was negatively correlated with BG, CBH, AP, and BX activities, suggesting that these taxa were not the direct agents of enzyme production. Chytridiomycota are often pathogenic on plants (Parker

1985), and animals (Longcore et al. 1999), which may negatively affect the abundance other soil organisms, leading to lower EEA. However, because Chytridiomycota comprised a limited amount of sequences in our dataset, it may be more likely that the relative abundance of Chytridiomycota inversely co-varied with the relative abundance of other saprotrophic fungi (i.e. Ascomycota and Basidiomycota), which caused this trend.

The ability of fungal communities to affect soil EEA may be greater in other systems. For example, other studies from forest soils have indicated that shifts in fungal phyla are correlated with soil EEA (Waldrop et al. 2000; Burke et al. 2011; Rinkes et al. 2011). In genomic and proteomic studies, fungi differ in their capability to produce different enzymes (Schneider et al. 2012; Baldrian et al. 2012). However, these studies occurred at single locations where environmental parameters vary less than in our regional study. Indeed, in our study region, abiotic factors varied by orders of magnitude between sampling sites while beta-diversity of fungal community composition was not as variable (Kivlin and Treseder unpublished data). This trend could lead to EEA correlating with abiotic factors more than fungal composition.

There are several nonexclusive mechanisms that may explain why species-level fungal composition does not vary with EEA in our study. First, bacteria, archaea, and plants may contribute a substantial portion of soil enzymes in our region. Second, soil extracellular enzymes may be produced in relatively equal amounts by all fungi and their activities may be mostly regulated by soil nutrient or climatic conditions. The fungi we detected by DNA sequencing may also not be active or producing soil extracellular enzymes. Finally, soil extracellular enzymes can become stabilized in the soil matrix and therefore be produced by taxa that were not present in the sampled community. Future work addressing the relative importance of these mechanisms is warranted.

Our study only represents a portion of the variables affecting EEA in natural systems. For example, we did not measure environmental variables such as soil texture, total soil P, soil metal concentrations or plant community composition, all of which may affect soil EEA. The environmental variables that we measured only spanned a relatively small range. For instance, soil pH in our study only ranged from 6.1 to 7.9 while other studies have found larger shifts in microbial composition in soils ranging from pH 4 to 8 (Rousk et al. 2009, 2010). Therefore, our findings are most useful for interpreting factors potentially influencing EEA at the regional scale, and our ability to make global predictions is limited. Furthermore, the relative abundance of fungal taxa represented in 454 pyrosequencing data is largely biased. However, this bias may only affect our adonis models, not the multiple regression models or dbRDA, as the largest bias often occurs at taxonomic resolutions smaller than the phylum level (Amend et al. 2010).

Nevertheless, the dbRDA models explained a majority of the variance for most of the enzymes in our study. The large influence of abiotic variables on EEA in both the dbRDA and multiple regression models indicates that EEA may be altered in future climates and global change conditions. In particular, PPO activities may decline if droughts become more prevalent, as are expected in this region (IPCC 2007). Furthermore, BG, CBH, AG, AP, BX and PPO activity may increase if predicted N deposition from the Los Angeles basin affects total N concentrations in soils (Fenn et al. 2003). The interplay of these two contrary effects may determine ecosystem-level carbon cycling rates in the future.

Climate change and N deposition are expected to be widespread, and affect EEA at multiple scales. Therefore, incorporating the relationships between climate, soil nutrient concentrations and EEA into global change models has the potential to enhance predictions of global decomposition rates. Including phylum-level shifts in fungal composition and changes in fungal biomass may also increase the predictive power of biogeochemical models, as these factors explained almost a third of the variance in EEA in our study. If fungal communities differ substantially between locations, (i.e. Tedersoo et al. 2012) then this variation may also correspond to large shifts in soil EEA (Strickland et al. 2009). Ultimately, the inclusion of abiotic factors, broadly classified microbial community composition, and seasonal variability may yield the most predictive decomposition models.

Acknowledgments C.V. Hawkes, S.R. Holden, C. Averill, B.G. Waring, H.E. Giauque and E.R. Worchel provided insightful comments on earlier drafts of this manuscript. C. Doan and A. Chan assisted in EEA measurements. This publication was developed under STAR Fellowship Assistance Agreement no. FP917191-01-0 awarded by the U.S.

Environmental Protection Agency (EPA) to SNK. It has not been formally reviewed by the EPA. The views expressed in this publication are solely those of the authors and EPA does not endorse any products or commercial services mentioned. SNK and KKT were supported by a Kearney Foundation grant and a National Science Foundation Ecosystems grant.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19(6):716–723

Allison S, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37(5):937–944

Allison SD, Lebauer DS, Ofrecio MR, Reyes R, Ta A-M, Tran TM (2009) Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biol Biochem 41(2):293–302

Amend AS, Seifert KA, Bruns TD (2010) Quantifying microbial communities with 454 pyrosequencing: does read abundance count? Mol Ecol 19(24):5555–5565

Averill C, Finzi A (2011) Plant regulation of microbial enzyme production in situ. Soil Biol Biochem 43(12):2457–2460

Baldrian P (2008) Enzymes of saprotrophic basidiomycetes, vol Chapter 2, Vol 28., British mycological society symposia seriesElsevier, Amsterdam, pp 19–41

Baldrian P, Kolarik M, Stursova M, Kopecky J, Valaskova V, Vetrovsky T, Zifcakova L, Snajdr J, Ridl J, Vlcek C, Voriskova J (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J 6(2):248–258

Boerner REJ, Brinkman JA, Smith A (2005) Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biol Biochem 37(8):1419–1426

Brockett BFT, Prescott CE, Grayston SJ (2012) Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol Biochem 44(1):9–20

Brown JKM, Hovmoeller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537–541

Brundett M, Piche Y, Peterson R (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can J Bot 62:2128–2134

Burke DJ, Weintraub MN, Hewins CR, Kalisz S (2011) Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. Soil Biol Biochem 43(4):795–803

Burns RG (1978) Soil enzymes. Academic, New York

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26(2): 266–267

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010b) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337(6098):1084–1087

Choi JH, Kang H, Park SS (2009) Comparison of enzyme activities in vegetated and nonvegetated sediments. J Environ Eng 135(5):299–305

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235–252

DeForest J, Zak DR, Pregitzer KS, Burton AJ (2004) Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Sci Soc Am J 68(1):132–138

DeForest J, Smemo K, Burke D, Elliott H, Becker J (2012) Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. Biogeochemistry 109(1):189–202

Drake JE, Darby BA, Giasson MA, Kramer MA, Phillips RP, Finzi AC (2012) Stoichiometry constrains microbial response to root exclusion—insights from a model and a field experiment in a temperate forest. Biogeosci Discuss 9(6):6899–6945

Dunn OJ (1961) Multiple comparisons among means. J Am Stat Assoc 56:52–64

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19):2460–2461

Fenn M, Baron J, Allen E, Rueth H, Nydick K, Geiser L, Bowman W, Sickman J, Meixner T, Johnson D, Neitlich P (2003) Ecological effects of nitrogen deposition in the western United States. BioSci 53(4):404–420

Fernandez-Calvino D, Rousk J, Brookes PC, Baath E (2011) Bacterial pH-optima for growth track soil pH, but are higher than expected at low pH. Soil Biol Biochem 43(7): 1569–1575

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103: 626–631

Frankenberger WT Jr, Johanson JB (1982) Effect of pH on enzyme stability in soils. Soil Biol Biochem 14(5):433–437

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. For Ecol Manag 196(1):159–171

German DP, Marcelo KRB, Stone MM, Allison SD (2012) The Michaelis–Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. Glob Change Biol 18(4):1468–1479

Hattenschwiler S, Tiunov A, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36:191–218

Henry HAL, Juarez JD, Field CB, Vitousek PM (2005) Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density

fractionation in a California annual grassland. Glob Change Biol 11(10):1808–1815
Hudson BD (1994) Soil organic matter and available water capacity. J Soil Water Conserv 49(2):189–194
IPCC (2007) Climate change 2007: the physical science basis, summary for policymaders
Joergensen RG, Wichern F (2008) Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol Biochem 40(12):2977–2991
Kivlin SN, Hawkes CV, Treseder KK (2011) Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biol Biochem 43(11):2294–2303
Kourtev PS, Ehrenfeld JG, Haggblom M (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biol Biochem 35(7):895–905
Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40(9):2407–2415
Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol Monogr 69(1):1–24
Leprince F, Quiquampoix H (1996) Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Eur J Soil Sci 47(4):511–522
Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a Chytrid pathogenic to amphibians. Mycologia 91(2):219–227
Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecol Monogr 76(2):151–174
Parker MA (1985) Local population differentiation for compatibility in an annual legume and its host-specific fungal pathogen. Evolution 39(4):713–723
Parker SS, Schimel JP (2011) Soil nitrogen transformations differ between the summer and growing season in California grassland. Appl Soil Ecol 48(2):185–192
Peay KG, Schubert MG, Nguyen NH, Bruns TD (2012) Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. Mol Ecol 16:4122–4136
R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Reeder J, Knight R (2010) Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. Nat Methods 7(9):668–669
Rinkes ZL, Weintraub MN, DeForest JL, Moorhead DL (2011) Microbial substrate preference and community dynamics during decomposition of *Acer saccharum*. Fungal Ecol 4(6):396–407
Robertson GP, Coleman DC, Bledsoe C, Sollins P (1999) Standard soil methods for long-term ecological research. Oxford University Press, New York
Rousk J, Brookes PC, Baath E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. App Environ Microbiol 75(6):1589–1596
Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4(10):1340–1351
Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol Biochem 34(9):1309–1315
Schneider T, Gerrits B, Gassmann R, Schmid E, Gessner MO, Richter A, Battin T, Eberl L, Riedel K (2010) Proteome analysis of fungal and bacterial involvement in leaf litter decomposition. Proteomics 10(9):1819–1830
Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME J 6(9): 1749–1762
Sinsabaugh RL, Moorhead D (1994) Resource allocation to extracellular enzyme production—a model for nitrogen and phosphorus control of litter decomposition. Soil Biol Biochem 26(10):1305–1311
Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11(11): 1252–1264
Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462(7274):795–798
Stone MM, Weiss MS, Goodale CL, Adams MB, Fernandez IJ, German DP, Allison SD (2012) Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Glob Change Biol 18:1173–1184
Strickland MS, Rousk J (2010) Considering fungal:bacterial dominance in soils—methods, controls, and ecosystem implications. Soil Biol Biochem 42(9):1385–1395
Strickland MS, Lauber CL, Fierer N, Bradford MA (2009) Testing the functional significance of microbial community composition. Ecology 90(2):441–451
Talbot JM, Treseder KK (2012) Interactions among lignin, cellulose, and nitrogen drive litter chemistry-decay relationships. Ecology 93(2):345–354
Tedersoo L, Bahram M, Toots M, DiÉDhiou AG, Henkel TW, KjØLler R, Morris MH, Nara K, Nouhra E, Peay KG, PÕLme S, Ryberg M, Smith ME, KÕLjalg U (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Mol Ecol 21(17):4160–4170
Toberman H, Evans CD, Freeman C, Fenner N, White M, Emmett BA, Artz RRE (2008) Summer drought effects upon soil and litter extracellular phenol oxidase activity and soluble carbon release in an upland Calluna heathland. Soil Biol Biochem 40(6):1519–1532
Turner BL (2010) Variation in pH optima of hydrolytic enzyme activities in tropical ran forest soils. Appl Environ Microbiol 76(19):6485–6493
Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32(13):1837–1846

Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Glob Change Biol 15(7):1631–1639

Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154(2):327–338

Zak DR, Holmes WE, MacDonald NW, Pregitzer KS (1999) Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. Soil Sci Soc Am J 63(3):575–584